Simultaneous monitoring of transcription and translation in mammalian cell-free expression (CFE) in bulk and in cell-sized droplets

Shue Wang[1], Sagardip Majumder[1], Nicholas J. Emery[1,2], Allen P. Liu[1,3,4,5,*]

[1]Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI, USA.

[2]Present address: Department of Biomedical Engineering, Boston University, Boston, MA, USA.

[3]Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA.

[4]Cellular and Molecular Biology Program, University of Michigan, Ann Arbor, MI, USA.

[5]Biophysics Program, University of Michigan, Ann Arbor, MI, USA.

*Corresponding author: Allen Liu, Ph.D., University of Michigan, Ann Arbor, 2350 Hayward Street, MI 48105. Tel: 734-764-7719, Email: allenliu@umich.edu

Figure S1. Stability of double-stranded LNA probe at different pH values and different incubation times.

Figure S2. Fluorescence intensity of LNA probe with different CFE components.

Figure S3. mRNA and protein synthesis dynamics with and without translation inhibitor cycloheximide (CHX).

Figure S4. The effect of LNA probe on GFP expression in HeLa-based CFE.

Figure S5. Standard calibration curve of GFP.

Figure S6. Detection of mRNA and protein expression in HeLa-based CFE.

Figure S7. The effect of pre-incubation of HeLa lysate on DNA template.

Figure S8. A computational model for mRNA and protein synthesis.

Figure S9. Images of a single emulsion droplet without encapsulating DNA plasmid.

Table S1. LNA probes and synthetic DNA target sequences.

Table S2. pT7-CFE-GFP sequence

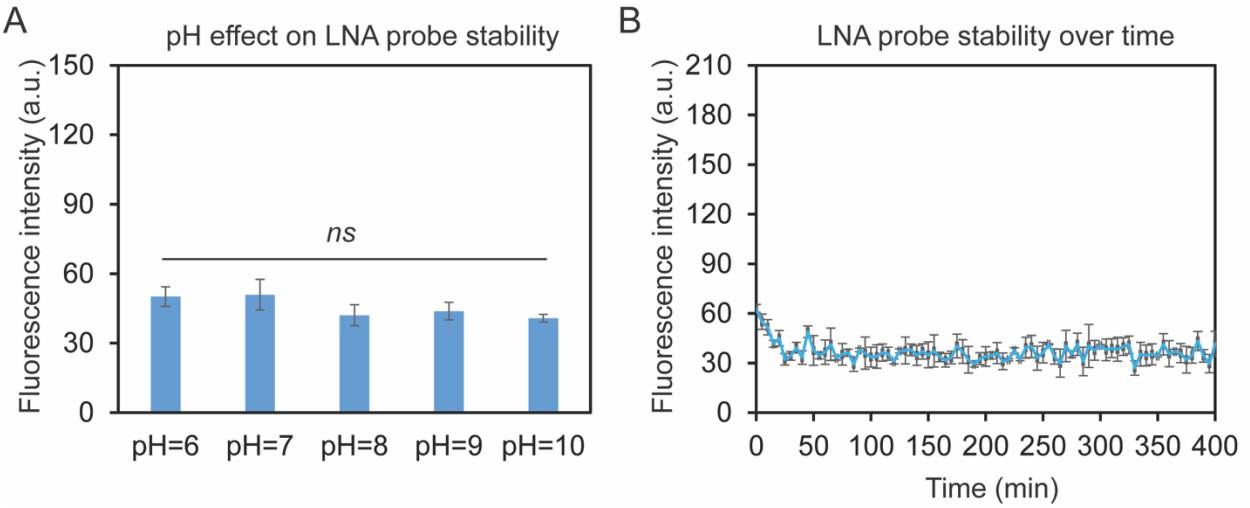

Figure S1. Stability of double-stranded LNA probe at different buffer (Tris-EDTA, TE buffer) pH values and different incubation times. (A) Fluorescence of the LNA probe in TE buffer at different pH values for 4 hours. (B) The stability of double-stranded probe over the course of 400 minutes. Data are shown as mean ± s.e.m. (n = 3).

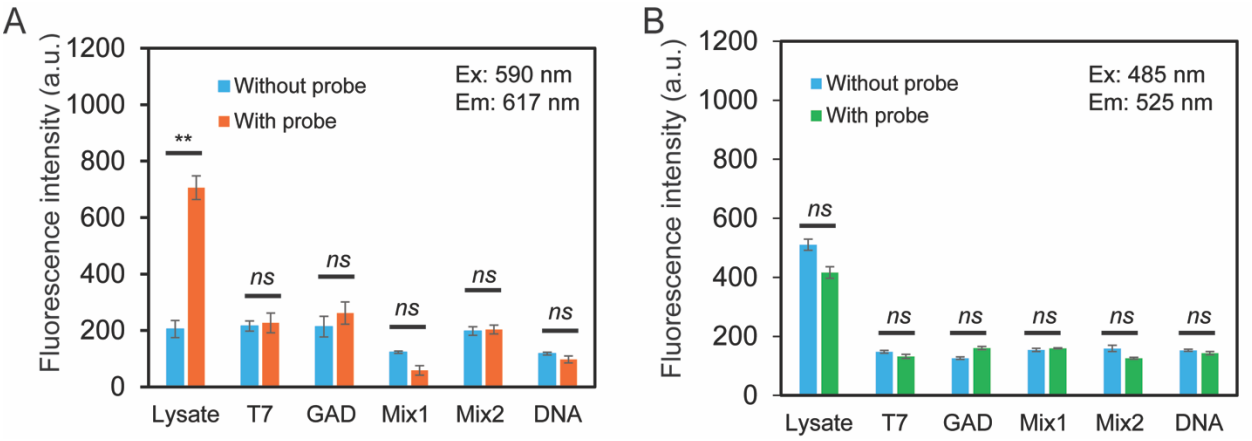

Figure S2. Background fluorescence intensity of double-stranded LNA probe with individual CFE components. (A) Fluorescence intensity measured in each CFE component with and without LNA probe with an excitation wavelength of 590 nm and emission wavelength of 617 nm. (B) Fluorescence intensity measured in each CFE component with and without LNA probe with an excitation wavelength of 485 nm and emission wavelength of 525 nm. The samples were prepared and incubated at 32 °C for 4 hours before measurements were taken. Data are shown as mean ± s.e.m. (n = 3; ** p <0.001; unpaired Student's t-test).

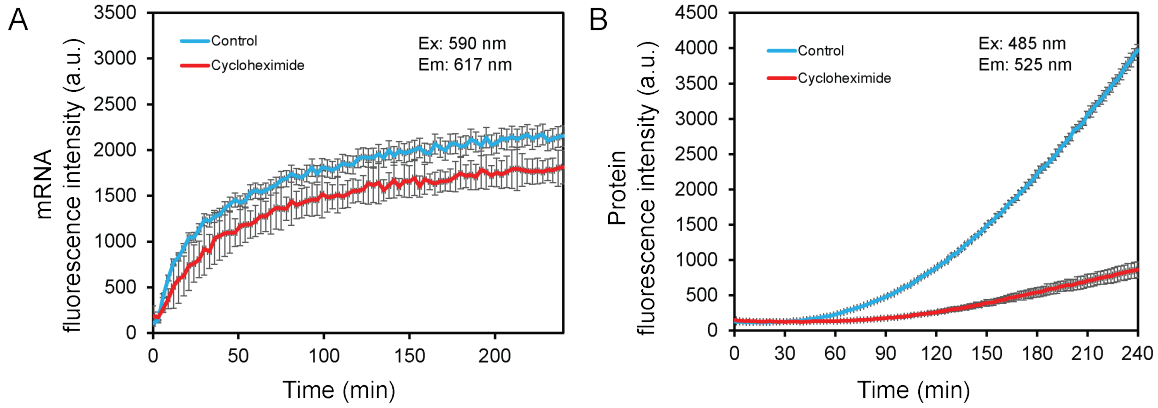

Figure S3. mRNA and protein synthesis dynamics with and without translation inhibitor cycloheximide (CHX). (A) mRNA synthesis dynamics with and without CHX. (B) Protein synthesis dynamics with and without CHX. CHX was dissolved in ethanol at the concentration of 150 μM. The final concentration of CHX in the experiments was 150 nM. An equal amount of ethanol was added to the control group. The DNA and probe concentrations used were 2 nM and 100 nM, respectively. Data are representatives of two independent experiments in triplicate. Data are shown as mean ± s.e.m.

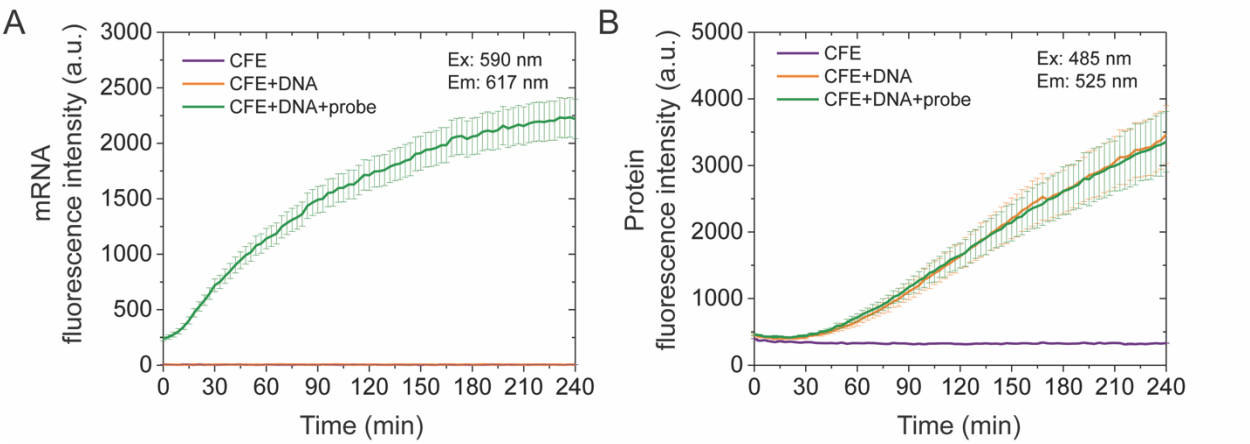

Figure S4. The effect of LNA probe on GFP expression in HeLa-based CFE. (A) Comparison of mRNA fluorescence intensity in CFE only, CFE with DNA, and CFE with DNA and probe. (B) Comparison of GFP fluorescence intensity in CFE only, CFE with DNA, and CFE with DNA and probe. The samples were prepared in a volume of 10 µL in a 96-well plate and measured on a fluorescence plate reader with an excitation wavelength of 485 nm and emission wavelength of 525 nm for (A), and excitation wavelength of 590 nm and emission wavelength of 617 nm for (B). The DNA and probe concentrations used were 2 nM and 100 nM, respectively. Data are representatives of three independent experiments. Data are shown as mean ± s.e.m.

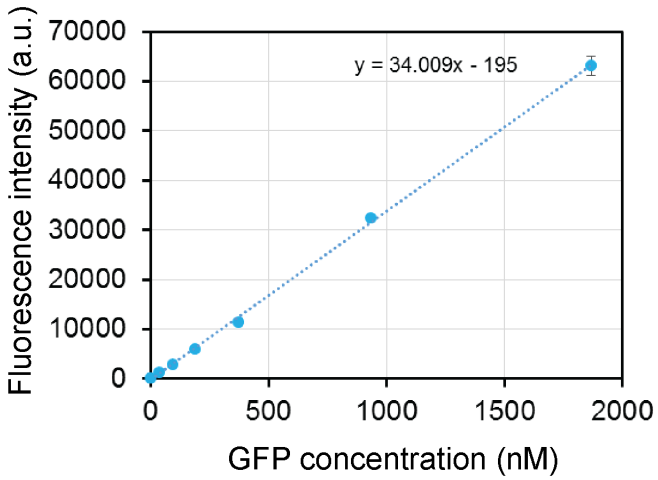

Figure S5. Standard calibration curve of GFP. The standard curve was produced by measuring the fluorescence of varying concentrations of GFP in a microplate reader using excitation and emission wavelengths of 485 and 525 nm, respectively. Experiments were performed three times independently with triplicates. Data are shown as mean ± s.e.m. (n = 3).

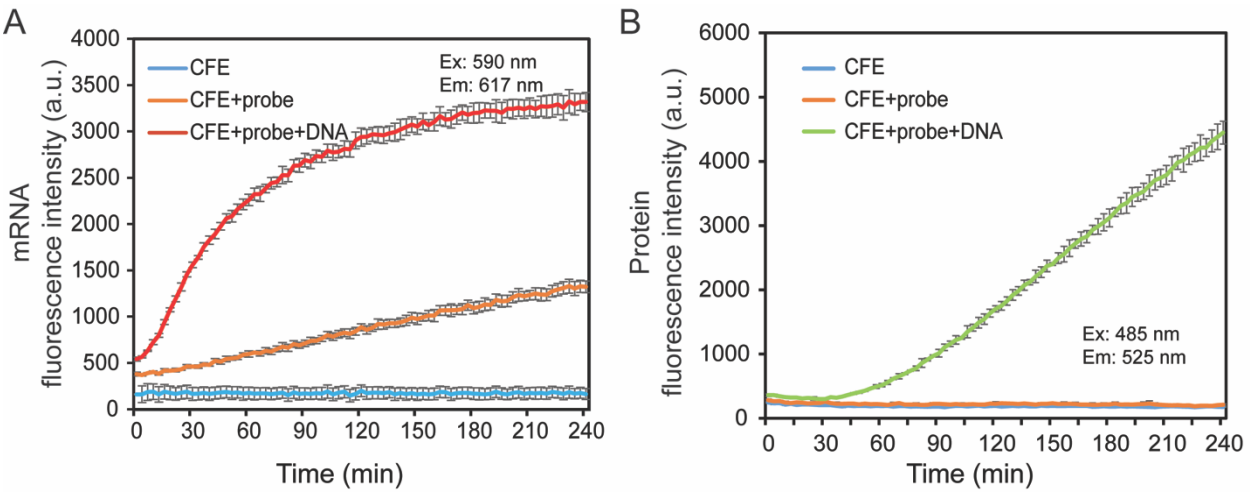

Figure S6. Detection of mRNA and protein expression in HeLa-based CFE. (A) Comparison of mRNA fluorescence intensity in CFE, CFE and probe, CFE with DNA and probe. (B) Comparison of protein fluorescence intensity in CFE, CFE and probe, CFE with DNA and probe. The DNA concentration was 2 nM. The probe concentration used in this experiment was 100 nM. Experiments were repeated independently three times. Data are expressed as mean ± s.e.m.

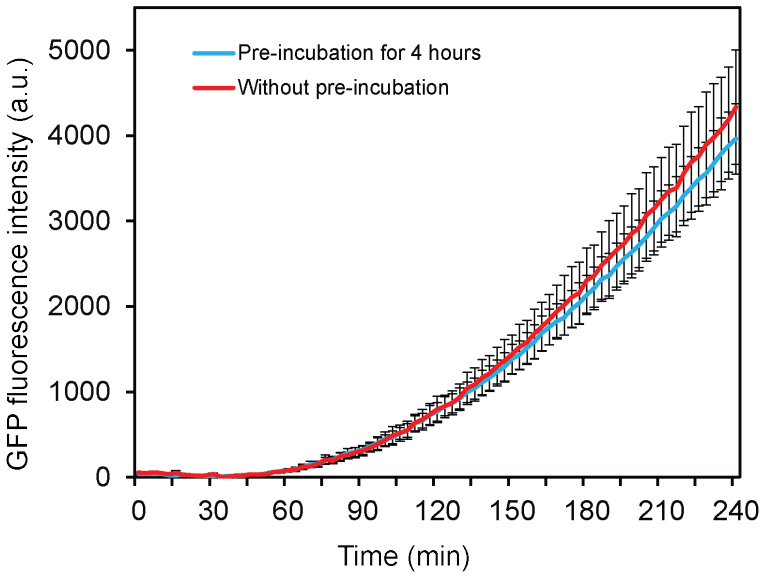

Figure S7. The effect of pre-incubation of HeLa lysate on DNA template. For the pre-incubation test, HeLa lysate and pT7-CFE-GFP template were pre-incubated at 32°C for 4 hours and other components were added. The synthesized GFP protein were monitored by measuring green fluorescence intensity. The samples were prepared in a 96-well plate and measured on a fluorescence plate reader with an excitation wavelength of 485 nm and emission wavelength of 525 nm. The DNA and probe concentrations used were 2 nM. Data are representatives of two independent experiments in triplicate. Data are shown as mean ± s.e.m.

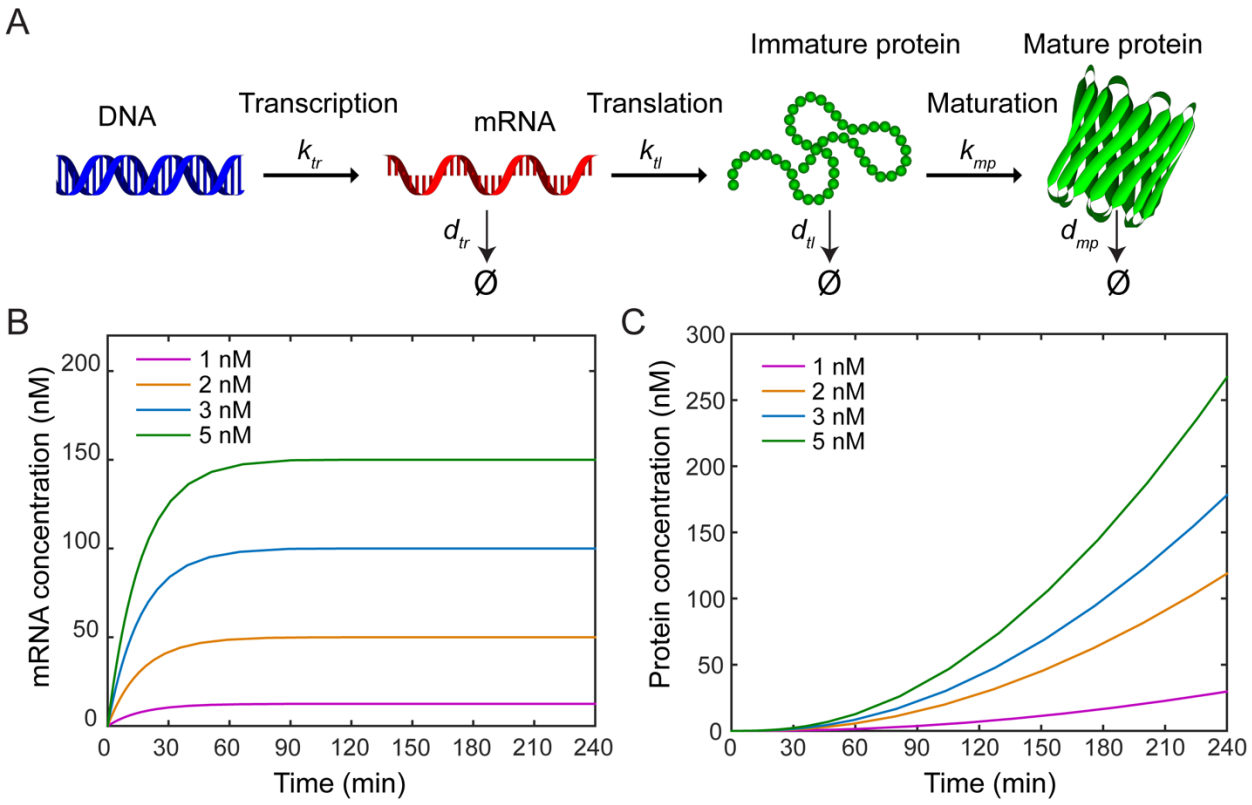

Figure S8. A computational model for mRNA and protein synthesis. (A) The model describes the gene expression dynamics including transcription, translation, and protein maturation. (B) Simulation results of mRNA synthesis in CFE at different DNA concentrations. (C) Simulation results of protein synthesis in CFE at different DNA concentrations (k_{tl}=1.5 nM/min, k_{tr}=0.06 nM/min, k_{mp}=0.008 nM/min). The transcription rate and translation rate were determined based on the experimental data. First, the mRNA and protein levels were quantified by measuring average fluorescence intensity using the LNA probe. A measurement of the relative amount of labeled mRNAs and proteins over time revealed the time scale of mRNA synthesis and protein synthesis. In this model, the degradation rates were set to zero (d_{tr}= d_{tl} = d_{mp} =0).

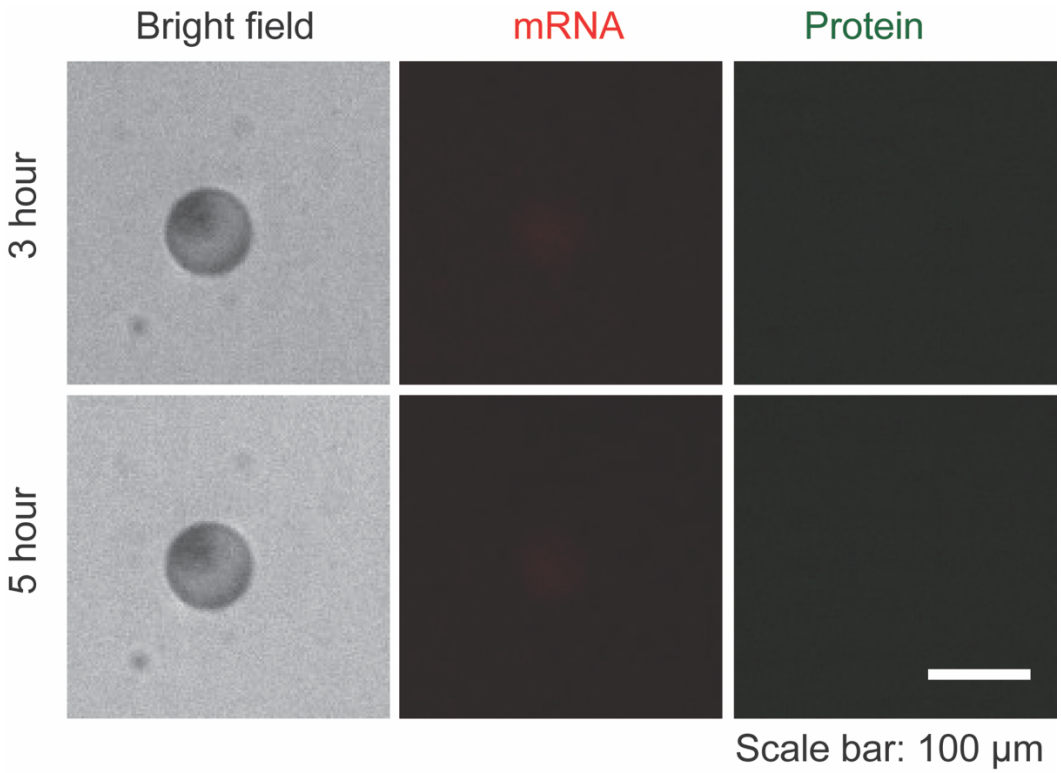

Figure S9. Images of single emulsion droplet without encapsulating DNA plasmid. CFE and LNA probe were encapsulated in droplets and imaged at 3 hours and 5 hours. Data are representative images from three independent experiments. Scale bar: 100 μm.

Table S1. LNA probes and synthetic DNA target sequences

	Sequence (5'-3')	Fluorophore	Free Energy (ΔG)
Probe	+TC+AC+CT+TG+AA+GT+CG+CC+GA+TCA	/5' Texas Red	-26.99 kcal/mol
Quencher	+TT+CA+AG+GT+GA	/3' Iowa Black	-9.75 kcal/mol
Target	TGATCGGCGACTTCAAGGTGA		

* + represents LNA monomer

Table S2. pT7-CFE-GFP sequence

Plasmid pT7-CFE-GFP
TAATACGACTCACTATAGGGCGAATTAATTCCGGTTATTTTCCACCATATTGCCGT CTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTC CTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGA AGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCC TTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAG CCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTG AGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCACCTCAAGCGTATTCAACAAG GGGCTGAAGGATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCC TCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCC CGAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATGATAATATGGCCACC ACCCATATGATGGAGAGCGACGAGAGCGGCCTGCCCGCCATGGAGATCGAGTG CCGCATCACCGGCACCCTGAACGGCGTGGAGTTCGAGCTGGTGGGCGGCGGA GAGGGCACCCCCGAGCAGGGCCGCATGACCAACAAGATGAAGAGCACCAAAGG CGCCCTGACCTTCAGCCCCTACCTGCTGAGCCACGTGATGGGCTACGGCTTCTA CCACTTCGGCACCTACCCCAGCGGCTACGAGAACCCCTTCCTGCACGCCATCAA CAACGGCGGCTACACCAACACCCGCATCGAGAAGTACGAGGACGGCGGCGTGC TGCACGTGAGCTTCAGCTACCGCTACGAGGCCGGCCGCGTGATCGGCGACTTC AAGGTGATGGGCACCGGCTTCCCCGAGGACAGCGTGATCTTCACCGACAAGAT CATCCGCAGCAACGCCACCGTGGAGCACCTGCACCCCATGGGCGATAACGATC TGGATGGCAGCTTCACCCGCACCTTCAGCCTGCGCGACGGCGGCTACTACAGC TCCGTGGTGGACAGCCACATGCACTTCAAGAGCGCCATCCACCCCAGCATCCTG CAGAACGGGGGCCCCATGTTCGCCTTCCGCCGCGTGGAGGAGGATCACAGCAA CACCGAGCTGGGCATCGTGGAGTACCAGCACGCCTTCAAGACACCGGATGCAG ATGCCGGTGAAGAACTCGAGCACCACCACCACCACCACTGAGATCTGACTGAAA AAAAAAAAAAAAAAAAAAAAANAAAAAGTTTAAACACTAGTCCGCTGAGCAATAAC TAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG

Yellow: T7 promoter and T7 terminator

Grey: IRES

Green: GFP

Light blue: probe sensing region